CLAIMS

1. A method for identifying ligands or aptamers specific for a membrane receptor protein-tyrosine kinase (RPTK), expressed in an activated or nonactivated form, by cells, using a mixture of nucleic acids, which method comprises at least the following steps:
   (a) bringing a mixture of nucleic acids into contact with cells not expressing said receptor protein-tyrosine kinase or expressing it in a nonactivated form (C_N cells), said cells having the same cell type as cells expressing the same receptor protein-tyrosine kinase but in an activated form, due to the existence of a mutation in the extracellular domain (C_{Te} cells);
   (b) recovering a first subset S1 of nucleic acids which do not bind to the C_N cells, in step (a);
   (c) bringing said first subset S1 into contact with C_i cells, having the same cell type as the C_{Te} cells, but expressing said receptor protein-tyrosine kinase mutated in its intracellular part, said C_i cells exhibiting a phenotype of the same type as that of the C_{Te} cells;
   (d) recovering a second subset S2 of nucleic acids which do not bind to the C_i cells in step (c);
   (e) bringing the second subset S2 into contact with the C_{Te} cells;
   (f) recovering the nucleic acids which bind to said C_{Te} cells, i.e. those exhibiting a high affinity with respect to the cells expressing said receptor protein-tyrosine kinase mutated in the extracellular domain, after dissociation of the cell-nucleic acid complexes;

(g) amplifying said nucleic acids with high affinity for the cells expressing said receptor protein-tyrosine kinase mutated in the extracellular domain, so as to obtain a mixture of nucleic acids, enriched in nucleic acids having a high affinity for said C_{Te} cells, and

(h) identifying the ligands or aptamers specific for the cells expressing receptor protein-tyrosine kinases (RPTKs) in an activated form, from the mixture obtained in (g).

2. The method as claimed in claim 1, characterized in that steps (a)-(g) are repeated using the mixtures enriched in ligands or aptamers from the preceding cycle, until at least one aptamer is obtained, the affinity of which, defined by its dissociation constant (Kd), can be measured and is suitable for pharmaceutical use.

3. The method as claimed in claim 1 or claim 2, characterized in that the starting nucleic acid combinatorial library contains at least 10^2 nucleic acids, preferably between 10^9 and 10^{15} nucleic acids, and advantageously consists of nucleic acids comprising random sequences comprising, respectively at their 5' and 3' ends, fixed sequences for PCR amplification, preferably the sequences SEQ ID NO:1 and SEQ ID NO:2 or a fragment of at least 8 nucleotides of these sequences.

4. The method as claimed in any one of claims 1 to 3, characterized in that said random sequences each contain between 10 and 1000 nucleotides, preferably 50 nucleotides, and are advantageously DNAs, RNAs or modified nucleic acids.

5. The method as claimed in any one of claims 1 to 4,

characterized in that the identification of the ligands or aptamers specific for the C_{Te} cells according to step (h) comprises an evaluation of the biological activity of said aptamers on said C_{Te} cells.

6. The method as claimed in any one of claims 1 to 5, characterized in that said biological activities which are advantageously evaluated are the following:

(a) inhibition or activation of the auto-phosphorylation of the RPTK,

(b) inhibition or activation of the kinase activation cascade,

(c) inhibition of the phosphorylation of the normal RPTK of C_N cells activated by suitable stimulation, and

(d) reversion of the phenotype associated with activation of the RPTK.

7. An aptamer, characterized in that it is specific for cells expressing a receptor protein-tyrosine kinase (RPTK) in an activated or nonactivated form and can be identified by means of the method for identifying aptamers as claimed in any one of claims 1 to 6.

8. The aptamer as claimed in claim 7, characterized in that it is specific for cells expressing a receptor protein-tyrosine kinase (RPTK) in an activated or nonactivated form, which RPTK is in particular selected from the group consisting of the following membrane receptors:
EGFR (Epithelial Growth Factor Receptor), InsulinR (Insulin Receptor), PDGFR (Platelet-derived Growth Factor Receptor), VEGFR (Vascular Endothelial Growth Factor Receptor), FGFR (Fibroblast Growth Factor Receptor), NGFR (Nerve Growth Factor Receptor), HGFR (Hepatocyte Growth Factor

Receptor), EPHR (Ephrin Receptor), AXL (Tyro 3 PTK), TIE (Tyrosine Kinase Receptor in endothelial cells), RET (Rearranged During Transfection), ROS (RPTK expressed in certain epithelial cells) and LTK (Leukocyte Tyrosine Kinase).

9. The aptamer as claimed in claim 7 or claim 8, characterized in that it recognises a Ret receptor in an activated form, and in particular the Ret receptor activated by mutation at a cysteine located in the extracellular domain, preferably at codons 609, 611, 618, 620 or 634.

10. The aptamer as claimed in claim 9, characterized in that it can be identified by means of the method comprising:

(a) bringing a mixture of nucleic acids into contact with C_N cells not expressing any Ret receptor in an activated form,

(b) recovering a first subset S1 of nucleic acids which do not bind to said C_N cells, in step (a),

(c) bringing said first subset S1 into contact with C_i cells expressing a Ret receptor, mutated in its intracellular domain, in particular the mutated receptor Ret^{M918T},

(d) recovering a second subset S2 of nucleic acids which do not bind to said C_i cells,

(e) bringing the second subset S2 into contact with C_{Te} cells expressing a Ret receptor activated by mutation in the extracellular domain, which receptor is selected from the group consisting of mutated Ret receptors carrying a mutation on one of the cysteines located in the extracellular domain, preferably at Cys609, Cys611, Cys618, Cys620 or Cys634, preferably the Ret^{C634Y} receptor,

(f) recovering the nucleic acids bound to said C_{Te} cells, i.e. exhibiting both a high affinity

and a binding specificity for the cells expressing a mutated Ret receptor as defined in step (e),

(g) amplifying said nucleic acids obtained in step (f), so as to obtain a mixture of nucleic acids, enriched in nucleic acids having a high affinity for the C_{Te} cells,

(h) repeating steps (a)-(g), until at least one aptamer is obtained, the affinity of which for the C_{Te} cells, defined by its dissociation constant (Kd), is measurable and suitable for a pharmacological activity, and

(i) identifying the aptamers specific for the cells expressing a Ret receptor in its activated form, selected from the mixture obtained in (h).

11. The aptamer as claimed in claim 10, characterized in that:

- the C_N cells are in particular wild-type PC12 cells (reference ECACC No. 88022) or wild-type NIH 3T3 cells (reference ECACC No. 93061524),
- the C_i and C_{Te} cells are obtained by introducing an oncogene bearing a mutation, respectively intracellular and extracellular, in C_N cells in culture in such a way that the latter express the oncogene.

12. An aptamer, characterized in that it can be obtained by means of a method of identification as defined in claims 1 to 11, and in that it is selected from the group consisting of the aptamers of formula (I):

$$R_1\text{-}R\text{-}R_2 \qquad (I),$$

in which:

R_1 represents 5' GGGAGACAAGAAUAAACGCUCAA 3' (SEQ ID NO:1) or a fragment of 1 to 23 nucleotides of

said SEQ ID NO:1;

R_2 represents 5' AACGACAGGAGGCUCACAACAGGA 3' (SEQ ID NO:2) or a fragment of 1 to 24 nucleotides of said SEQ ID NO:2, and

R represents a random sequence of 10 to 1000 nucleotides, preferably of 50 nucleotides.

13. The aptamer as claimed in claim 12, characterized in that R is preferably selected from the following sequences:

D4	5'GCGCGGGAAUAGUAUGGAAGGAUACGUAUACCGUGCAAUCCAGGGCAACG 3' (SEQ ID NO:3)
D12	5'GGGCUUCAUAAGCUACACCGGCCAACGCAGAAAUGCCUUAAGCCCGAGUU 3' (SEQ ID NO:4)
D14	5'GGCCAUAGCGCACCACCAAGAGCAAAUCCCUAAGCGCGACUCGAGUGAGC 3' (SEQ ID NO:5)
D20	5'GGGCCAAUCGAAGCCGGUAAUUCCCAAACUAACGUGCAAACUGCACCCGC 3' (SEQ ID NO:6)
D24	5'GCGGUAUGUAGGGAAUAGCACUUUUUUUGCGUAUACCUACACCGCAGCG 3' (SEQ ID NO:7)
D30	5'AGGCGAGCCCGACCACGUCAGUAUGCUAGACAACAACGCCCGCGUGGUAC 3' (SEQ ID NO:8)
D32	5'CCCCGCUUUUUGACGUGAUCGAACGCGUAUCAGUAACGUCAGCAGUCGAGC 3' (SEQ ID NO9)
D33	5'CAAAGCGUGUAUUCUCGUGAGCCGACCAUCGUUGCGAACAUCCCCGGAACG 3' (SEQ ID NO :10)
D42	5'GACCCGUAUGAAGGUGGCGCAGGACACGACCGUCUGCAAUGAGCGAGC 3' (SEQ ID NO:11)
D60	5'CCGACCUGUACAGCAGUUAGUUACACGUUUGAAACAACCGGCGUUCGAGC 3' (SEQ ID NO:12)
D76	5'GGCUUACACGGAGAAACAAGAGAGCGGCCCAAACUUGAUUGACAGUGGCC 3' (SEQ ID NO:13)
D71	5'GGCCCUUAACGCAAAAACGAAGGAUCAUCGAUUGAUCGCCUUAUGGGCU 3' (SEQ ID NO:14)
D87	5'CCGCGGUCUGUGGGACCCUUCAGGAUGAAGCGGCAACCCAUGCGGGCC 3' (SEQ ID NO:15)

14. The aptamer as claimed in claim 12 or claim 13, characterized in that the riboses of the purines bear a hydroxyl function on the carbon in the 2'-position, while the riboses of the pyrimidines bear a fluorine atom on the carbon in the 2'-position.

15. The aptamer as claimed in any one of claims 12 to 14, characterized in that it has one of the following sequences: SEQ ID NOs:31-33.

16. The aptamer as claimed in any one of claims 12 to 14, characterized in that it has formula II below: $5'R_4X_6X_5X_4X_3GGAAUAGX_2X_1R_3X'_1X'_2CGUAUACX'_3X'_4X'_5X'_6R_5 3'$ (II), the secondary structure of which is represented in figure 10, and in which:

- the riboses of the purines bear an OH group in the 2'-position and the riboses of the pyrimidines bear a fluorine atom in the 2'-

position,

- **R_3** is present or absent and represents an apical bulge (or loop) comprising:
  - a linear or branched carbon chain selected from the group consisting of C_6-C_{30} alkyl groups or C_6-C_{30} aryl groups;
  - a polymer such as PEG or PEI, or the like;
  - functional groups such as biotin, streptavidin, peroxidase;
  - other molecules of interest such as, for example, active ingredients, labeling tags, in particular fluorescent tags, or chelating agents for radioisotopes;
  - a natural or modified nucleotide sequence;

  preferably, R_3 represents the following bulges or loops (1) to (4):

  loop (1): 5' UGGAAGGA 3' (SEQ ID NO:29)

  loop (2): 5' CUUUUUU 3' (SEQ ID NO:30)

  loop (3): 5' GNPuA 3'

  loop (4): 5' UNCG 3',

  in which the riboses of the purines bear a hydroxyl function on the carbon in the 2'-position, while the riboses of the pyrimidines bear a fluorine atom on the carbon in the 2'-position,
- **X_1, X'_1, X_2, X'_2, X_3, X'_3, X_4, X'_4, X_5, X'_5, X_6 and X'_6** represent Py or Pu with, preferably:

  X_1-X'_1 corresponding to C-G, A-U, G-C or U-A

  X_2-X'_2 corresponding to C-G, A-U, G-C or U-A

  X_3-X'_3 corresponding to C-G, A-U, G-C or U-A

  X_4-X'_4 corresponding to C-G, A-U, G-C or U-A

  X_5-X'_5 corresponding to C-G, A-U, G-C or U-A

  X_6-X'_6 corresponding to C-G, A-U, G-C or U-A

  N corresponding to G or C or A or U,

  Pu corresponding to G or A, in which the riboses bear an OH group in the 2'-position,

  Py corresponds to U or C, in which the riboses bear a fluorine atom in the 2'-position, and
- **R_4** and **R_5** are present or absent and represent:

- a natural or modified nucleotide sequence, comprising between 1 and several thousand nucleotides, preferably between 1 and 39 nucleotides; a part of said nucleotide sequence or said sequence preferably comprising one of the following sequences:
  R_4 :
  5'-R_1-Z_1-3', with Z_1=G:
  5' GGGAGACAAGAAUAAACGCUCAAG 3' (SEQ ID NO:18)
  or
  5'-R_1-Z_1-3', with Z_1=GCGGUAU (SEQ ID NO:26):
  5' GGGAGACAAGAAUAAACGCUCAAGCGGUAU (SEQ ID NO:19), and
  R_5 :
  5'-Z_2-R_2-3', with Z_2=CAAUCCAGGGCAACG (SEQ ID NO:27):
  5' CAAUCCAGGGCAACGAACGACAGGAGGCUCACAACAGGA 3' (SEQ ID NO:20) or
  5'-Z_2-R_2-3', with Z_2=ACCGCAGCG (SEQ ID NO:28):
  5' ACCGCAGCGAACGACAGGAGGCUCACAACAGGA 3' (SEQ ID NO:21),
  5' GGGAGACAAGAAUAAACGCUCAAG 3' (SEQ ID NO:18)
  or
  5' GGGAGACAAGAAUAAACGCUCAAGCGGUAU (SEQ ID NO:19), for R_4 and
  5' CAAUCCAGGGCAACGAACGACAGGAGGCUCACAACAGGA 3' (SEQ ID NO: 20) or
  5' ACCGCAGCGAACGACAGGAGGCUCACAACAGGA 3' (SEQ ID NO:21) for R_5;
- a linear or branched carbon chain selected from the group consisting of C_6-C_{30} alkyl groups or C_6-C_{30} aryl groups;
- a polymer such as PEG or PEI, or the like;
- functional groups such as biotin, streptavidin, peroxidase;
- other molecules of interest such as, for example, active ingredients, labeling tags, in particular fluorescent tags, or chelating agents for radioisotopes.

17. The aptamer as claimed in claim 16, characterized in that R_3 represents 5' UGGAAGGA 3' (loop (1)), R_4 represents SEQ ID NO:18 and R_5 represents SEQ ID NO:20, the aptamer exhibiting such a structure (family D4) has both properties of binding to said Ret receptor and properties of inhibition of the activity of said receptor.

18. The aptamer as claimed in claim 17, characterized in that it has the sequence SEQ ID NO:22.

19. The aptamer as claimed in claim 16, characterized in that R_3 represents 5' CUUUUUU 3' (loop (2)), 5' GNPuA 3' (loop (3)) or 5' UNCG 3' (loop (4)), R_4 comprises from 1 to 30 nucleotides selected from SEQ ID NO:19 or from 1 to 24 nucleotides selected from SEQ ID NO:18 and R_5 comprises from 1 to 33 nucleotides of SEQ ID NO:21 or from 1 to 39 nucleotides selected from SEQ ID NO:20, the aptamer exhibiting such a structure having only properties of binding to said Ret receptor in its activated or nonactivated form, and in particular to the Ret receptor mutated in its extracellular domain.

20. The aptamer as claimed in claim 19, characterized in that R_3 represents 5' CUUUUUU 3' (loop (2)), R_4 represents SEQ ID NO:19 and R_5 represents SEQ ID NO:21.

21. The aptamer as claimed in claim 19 or claim 20, characterized in that it has SEQ ID NO:25.

22. The aptamer as claimed in claim 16, characterized in that R_3 represents 5' UGGAAGGA 3' (loop (1)), R_4 and R_5 are absent, the aptamer exhibiting such a structure having only properties of binding to said Ret receptor in its activated or nonactivated

form, and in that it has the sequence SEQ ID NO:23.

23. A reagent for diagnosing a tumor, characterized in that it consists of an aptamer as claimed in any one of claims 12 to 22.

24. The reagent as claimed in claim 23, characterized in that it corresponds to an aptamer of formula II:
$5'R_4X_6X_5X_4X_3GGAAUAGX_2X_1R_3X'_1X'_2CGUAUACX'_3X'_4X'_5X'_6R_53'$, in which R_3, R_4 and R_5 are absent.

25. The reagent as claimed in claim 24, characterized in that it corresponds to an aptamer of sequence:
5' GUAGGGAAUAGCACGUAUACCUAC 3' (SEQ ID NO:24).

26. The reagent as claimed in claim 23, characterized in that it corresponds to an aptamer of formula II, in which R_3 represents 5' CUUUUUU 3' and in that it corresponds to the sequence SEQ ID NO:25.

27. A reagent for diagnosing or detecting the Ret receptor in an activated or nonactivated form, characterized in that it consists of at least one aptamer as claimed in any one of claims 12 to 22.

28. A medicament, characterized in that it comprises an aptamer as claimed in any one of claims 7 to 22, which has both an ability to bind to an RPTK receptor and an inhibitory action with respect to said receptor in an activated form.

29. A medicament for use in the treatment of a tumor, characterized in that it comprises an aptamer as claimed in any one of claims 7 to 22, which has both an ability to bind to an activated RPTK receptor, and in particular to the receptor mutated in the extracellular domain, and in

particular to the Ret receptor mutated at one of the cysteines located in the extracellular domain (codons 609, 611, 618, 620 and 634), and an inhibitory action with respect to this mutated receptor.

30. The medicament as claimed in claim 28 or claim 29, characterized in that it corresponds to an aptamer of the aptamer family D4, as defined in claim 13, 16 or 17.

31. A pharmaceutical composition, characterized in that it comprises an aptamer as claimed in any one of claims 7 to 22, which has both an ability to bind to an RPTK receptor and an inhibitory action with respect to said receptor in its activated form.

32. A pharmaceutical composition, characterized in that it comprises:

- an aptamer as claimed in any one of claims 7 to 22, which has both an ability to bind to an activated RPTK receptor, and in particular to a receptor mutated in the extracellular domain, and in particular to the Ret receptor mutated at one of the cysteines located in the extracellular domain (codons 609, 611, 618, 620 and 634), and an inhibitory action with respect to this mutated receptor,
- another anticancer molecule, and
- at least one pharmaceutically acceptable vehicle.

33. The use of an aptamer which has both an ability to bind to an RPTK receptor and an inhibitory action with respect to this RPTK receptor, for screening products which interact with the RPTK receptor and which may or may not inhibit it.

34. The use of an aptamer which has both an ability to bind to an activated RPTK receptor, and in particular to the Ret receptor mutated at one of the cysteines located in the extracellular domain (codons 609, 611, 618, 620 and 634), and an inhibitory action with respect to this activated RPTK receptor, for screening products which interact with said RPTK receptor.

35. A method for screening products which interact with an RPTK receptor or targets which form a complex with said RPTK in an activated or nonactivated form, which method is characterized in that it comprises:

- bringing cells expressing RPTKs in an activated or nonactivated form into contact with the substance to be tested,
- adding, under suitable conditions, an aptamer as claimed in any one of claims 7 to 22, before, at the same time as or after the substance to be tested,
- evaluating the competitive binding between the aptamer and the molecule to be tested (for example: by measuring radioactivity, fluorescence, luminescence, surface plasmon resonance, BRET, FRET, or any other technique for demonstrating a molecular interaction).

36. The method as claimed in claim 35, characterized in that, after identification of the substances which bind competitively with the aptamer to the cells exhibiting RPTKs, the effect of these substances on the biological activity of said cells can be evaluated in order to find substances which inhibit or activate said biological activities of the cells exhibiting RPTKs.